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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: TERMINAL-PHOSPHATE-LABELED NUCLEOTIDES AND METHODS OF USE

(57) Abstract: The present invention relates to improved methods of detecting a target using a labeled substrate or substrate analog. The methods comprise reacting the substrate or substrate analog in an enzyme-catalyzed reaction which produces a labeled moiety with independently detectable signal only when such substrate or substrate analog reacts. The present invention, in particular, describes methods of detecting a nucleic acid in a sample, based on the use of terminal-phosphate-labeled nucleotides as substrate for nucleic acid polymerases. The methods provided by this invention utilize a nucleoside polyphosphate, dideoxynucleoside polyphosphate, or deoxynucleoside polyphosphate analogue which has a colorimetric dye, chemiluminescent, or fluorescent moiety, a mass tag or an electrochemical tag attached to the terminal-phosphate. When a nucleic acid polymerase uses this analogue as a substrate, an enzyme-activatable label would be present on the inorganic polyphosphate by-product of phosphoryl transfer. Cleavage of the polyphosphate product of phosphoryl transfer *via* phosphatase leads to a detectable change in the label attached thereon. When the polymerase assay is performed in the presence of a phosphatase, there is provided a convenient method for real-time monitoring of DNA or RNA synthesis and detection of a target nucleic acid.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/02785

A. CLASSIFICATION OF SUBJECT MATTER

IPC: C12Q 1/68(2006.01)

USPC: 435/6,91.1,91.2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 91.1, 91.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN, Medline, Biosis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6,232,075 B1 (WILLIAMS) 15 May 2001 (15.05.2001), entire document.	1-4, 6-9, 12, 14-15, 23-30
Y		10
X	US 2003/0064366 A1 (HARDIN et al.) 03 April 2003 (03.04.2003), entire document.	1-9, 11-30
Y	T4 DNA Ligase. In : New England BioLabs Catalog. 1995 page 78.	10

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"

document member of the same patent family

Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International application No.

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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
 2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of any additional fees.
 3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
 4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-31
- Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/02785

BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-31, drawn to a method of detecting a target using a labeled substrate.

Group II, claim(s) 32 and 33, drawn to a labeled phosphate containing compound.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The methods of at least claim 1 is not joined to the labeled phosphate containing compound of at least claim 32. The method is drawn to detecting a target using a labeled substrate in an enzyme catalyzed reaction which produces a labeled polyphosphate. This is not a special technical feature which joins the claimed inventions because Williams (USPN 6,232,075) teach detecting a target using a labeled substrate in an enzyme catalyzed reaction which produces a labeled polyphosphate (see col. 3 lines 38-56, where enzyme catalyzed is the cleavage of the gamma phosphate. The gamma phosphate is labeled with a fluorophore moiety and the signal is only detectable once the gamma phosphate is cleaved and released). The method of at least claims 1 is not limited in scope so as to require the labeled phosphate containing compound of claim 1 and therefore the claims are not joined by a special technical feature